REMARKS

Applicants respectfully request reconsideration. Claims 46-71 were previously pending in this application with claims 46, 53, 59, and 65 being independent claims. No amendments have been made and no new matter has been added.

Applicants acknowledge the courtesies extended by Examiner Pellegrino during a telephone discussion with the undersigned attorney on March 30, 2007. The substance of the discussion is summarized in the remarks set forth below.

Applicants respectfully traverse each of the rejections presented in the Office Action of December 19, 2006 for reasons similar to those set forth in their prior responses of September 19, 2006, May 21, 2004 and December 23, 2003 and the declaration of Stephan N. Eldridge (hereafter the "Eldridge Declaration") submitted therewith, which are incorporated herein by reference.

Rejections Under 35 U.S.C. § 102

Rejections in View of Mulhauser

Claims 46, 47, 50, 54-56, 59-62, 65-68 and 71 stand rejected under 35 U.S.C. §102(b) as purportedly being anticipated by Mulhauser (U.S. patent No. 5,695,525). The Examiner contends that Mulhauser discloses (Figs. 4a, 4b) a surgical repair material comprising a fabric 34, a barrier layer 36 and an edge barrier 32. The Examiner further contends that Mulhauser illustrates (Figs. 2a, 2b, 3h) a frame structure located at the edge to form an edge barrier. Applicants respectfully traverse these rejections.

As previously pointed out, Mulhauser '525 is a family member of Mulhauser '246 (US 5,766,246) which was previously applied during prosecution of the claims and over which the claims were found to be patentable. Applicants respectfully assert that the claims patentably distinguish over Mulhauser '525 for at least the same reasons set forth in the prior responses.

Independent claims 46, 59 and 65 recite, *inter alia*, a prosthesis or repair fabric with an edge barrier that inhibits the formation of adhesions with tissue or organs thereto, wherein the edge barrier isolates or covers at least a portion of the edge of the fabric.

Mulhauser is directed to an implantable prosthesis 10, 30 having a mesh layer 12, 34 and a semi-rigid frame or ring 14, 32 supporting the mesh layer. (Mulhauser '525, Col. 3, lines 42-

53; col. 5, lines 24-29). In the embodiment shown in Figs. 4a-4b, the fabric extends outwardly beyond the frame or ring 32 such that the frame 32 does not cover or isolate a portion of the fabric edge. Consequently, the frame 32 is not an edge barrier as recited in the claims for at least this reason.

In the Office Action, the Examiner contends that:

> the support frame 32 clearly isolates a portion of the edge or covers a portion of the edge since the claims do not set forth what an edge is defined as. (Office Action, page 4).

The Examiner further contends that:

> Since the claims do not set forth any special definition of "edge" it can be construed that since Mulhauser's frame is at the outer periphery of the fabric it is covering or isolating the edge. (Office Action, page 4.).

Applicants respectfully asserts that one of ordinary skill in the art would readily understand the term "edge" to be a narrow surface extending between the major surfaces of the fabric based on the plain meaning of "edge" and further in view of the specification which is consistent with the plain meaning of the term. Additionally, the claims recite that the fabric includes opposing first and second sides and an edge extending between the first and second sides. Therefore, one of ordinary skill in the art would understand the meaning of "edge" as set forth in the claims and further recognize that the frame 32 shown in Figs. 4a-4b of Mulhauser does not cover or isolate a portion of the fabric edge.

Nevertheless, as indicated in the Office Action, Mulhauser does disclose a prosthesis (Figs. 2) which includes a frame that covers the edge of the mesh fabric. As shown in Figs. 2 and 3(h), the frame may be configured to extend over the mesh layer at both the peripheral edge of the mesh layer and the surface margin of the mesh layer adjacent the peripheral edge. However, as explained previously, Mulhauser does not teach or suggest that the frame 14, 32 has any type of adhesion inhibiting properties.

In the Office Action, the Examiner contends that the claimed physical properties are purportedly present in the prior art material (edge barrier) to some extent even though they are not explicitly recited. (Office Action, page 2). Based on this contention, the Examiner attempts to shift the burden to Applicants to show that the frame does not have adhesion resistant properties. (Office Action, pages 2-3). However, as indicated previously, this is not the proper test for establishing a rejection based on inherency. Rather, the burden lies with the Patent

Office to provide rationale or evidence that the Mulhauser frame inherently possesses the claimed adhesion resistant properties.

As explained in MPEP §2112(IV):

> The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish inherency of that result or characteristic. Citing *In re Rijckaert*, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993) (emphasis in original).
>
> To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.' Citing *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999).

In an apparent attempt to provide a rationale or evidence to establish that the Mulhauser frame is inherently adhesion resistant, the Examiner contends that the frame is solid or rigid to provide support to the fabric and thus would inhibit adhesions from forming. (Office Action, page 3). Thus, it appears that the Examiner contends that a solid or rigid frame is inherently adhesion resistant. Applicants respectfully disagree.

As discussed previously and again indicated during the telephonic discussion, Applicants previously submitted a declaration to show that the Mulhauser frame does not inherently possess adhesion resistant properties. (See Eldridge Declaration). Silicone or polypropylene materials, which Mulhauser discloses may be employed for the ring or frame, do not inherently or necessarily inhibit adhesions. The adhesion resistant properties of a soft tissue repair prosthesis are affected by various factors such as the surface texture and pore size of the material that forms the prosthesis or portions of the prosthesis. (See Eldridge Declaration, paragraph 9). Thus, a prosthesis may be either resistant to the formation of adhesions or promote tissue ingrowth and adhesions depending upon the particular structural characteristics of its material. (See Eldridge Declaration, paragraph 9). For example, a prosthetic material, including silicone, having a surface texture or porosity of approximately 10μm or more is susceptible to adhesions with tissue or muscle. (See Eldridge Declaration, paragraph 9).

Applicant encloses several references (Woodward, "The Tissue Response to Implants and Its Evaluation by Light Microscopy" and Boyers, "Reduction of postoperative pelvic adhesions in the rabbit with Gore-Tex surgical membrane") describing the surface texture and porosity characteristics of an implantable material as these characteristics relate to the adhesion resistance of the material. The Woodward reference (page 370, right column) discloses that surface irregularities as small as 10-15μm result in the development of giant cells as the principal cellular interface between the host and non-reactive implant. Thus, Woodward indicates that a material with surface irregularities of approximately 10μm or more is susceptible to adhesions with tissue or muscle. The Boyers reference discloses that an implant manufactured with a relatively large pore size encourages tissue attachment and the infiltration of fibers into its microstructure, while an implant having an average pore size less than or equal to 1μm minimizes cellular penetration and tissue attachment. (Boyers, page 1069, left column).

Mulhauser provides no teaching or suggestion as to any structural characteristics of the frame that would determine its adhesion resistant properties. The surface texture and porosity of a silicone frame (as well as a molded polypropylene frame) can vary depending on the specific design parameters of the mold used to form the frame. (See Eldridge Declaration, paragraph 10). Therefore, a molded silicone frame can promote tissue ingrowth and adhesions with tissue and muscle. (See Eldridge Declaration, paragraph 10). Thus, although the Mulhauser frame may be molded from a silicone material, this does not necessarily provide a frame that inhibits adhesions to tissue and muscle, such that one of ordinary skill in the art would not consider the Mulhauser frame, even if formed of silicone material, as necessarily being resistant to tissue ingrowth and adhesions to tissue and muscle. (See Eldridge Declaration, paragraph 10).

In the Office Action, the Examiner stated that:

> In response to applicant's argument that both Meier and Mulhauser fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., porosity less than 10μm) are not recited in the rejected claim(s). However, it should be noted that this limitation is not supported by the disclosure and thus the Examiner is entitled to give terms in a claim its plain meaning as interpreted by one of skill in the art. It is noted that the specification must clearly set forth the definition explicitly and with reasonable clarity, deliberateness, and precision. Exemplification is not an explicit definition. Even explicit definitions can be subject to varying interpretations. . . Since the disclosure fails to define any special porosity for the edge barrier,

> it can be said that since both Meier's and Mulhauser's edge barriers are solid materials and not an open structure they clearly can prevent adhesions. (Office Action, pages 4-5).

As explained during the telephonic discussion, Applicant is relying upon the limitation that the edge barrier "inhibits the formation of adhesions with sensitive tissue and organs thereto." As discussed above and set forth in the Eldridge Declaration, one of ordinary skill in the art understands and would interpret this limitation as requiring the edge barrier to have particular characteristics, including porosity and surface texture characteristics, which render the barrier adhesion resistant. As discussed with the Examiner, Mulhauser fails to disclose any particular structural characteristics for the frame, such as surface texture or porosity, that would enable one of ordinary skill in the art to conclude that the Mulhauser frame inherently inhibits the formation of adhesions thereto.

As also explained during the telephonic discussion, the specification clearly supports the claim limitation that the peripheral barrier inhibits the formation of adhesions thereto. Further, this feature is enabled by the specification which discloses several examples of materials that are adhesion resistant.

As indicated above, the Examiner contends that the Mulhauser frame is solid or rigid to provide support to the fabric and thus would inhibit adhesions from forming. Applicant respectfully asserts that nowhere does Mulhauser state that the frame is solid, although several cross-sectional views of the frame would appear to suggest that the frame is of a generally solid, as opposed to a hollow, construction. Nevertheless, as set forth in the Eldridge Declaration, nothing in the figures provide any indication that the frame is adhesion resistant. (See Eldridge Declaration, paragraph 11). As indicated above, the adhesion resistance of a material implanted in a body depends on the structural characteristics of the material, including surface texture and porosity of the material, and that tissue ingrowth can occur when the surface texture or porosity is approximately 10μm or more. (See Eldridge Declaration, paragraph 11). This amount of surface texture and porosity is microscopic and undetectable with the naked eye. (See Eldridge Declaration, paragraph 11). Thus, simply because the drawings in Mulhauser do not illustrate a rough surface or large pores, the drawings do not indicate that the frame is resistant to tissue ingrowth or adhesions. (See Eldridge Declaration, paragraph 11).

Moreover, a solid structure does not inherently possess adhesion resistant properties as such structures may still have a porosity and/or surface texture that allows tissue ingrowth and

adhesion thereto. For example, a solid bar of chocolate typically has pores that are readily visible to the naked eye. The Examiner has established no basis to support the contention that a "solid" or "rigid" material is inherently adhesion resistant.

In view of the foregoing, claims 46, 59 and 65 patentably distinguish over Mulhauser, such that the rejections under §102 should be withdrawn. Mulhauser does not disclose an adhesion resistant edge barrier as recited in each of the claims.

Claims 47 and 50, claims 60-62, and claims 66-68 and 71 respectively depend from claims 46, 59 and 65 and are patentable for at least the same reasons. It is unclear as to the basis for the rejection of claims 54-56 as they depend from claim 53 which has not been rejected in view of Mulhauser. Nevertheless, claim 53, which also recites an edge barrier, patentably distinguishes over Mulhauser for at least the same reasons as independent claims 46, 59 and 65, and claims 54-56 are patentable for at least the same reasons. Accordingly, withdrawal of these rejections is respectfully requested.

Rejections in View of Meier

Claims 53, 56, 65, 68 and 71 stand rejected under 35 U.S.C. §102(b) as purportedly being anticipated by Meier (U.S. patent No. 3,416,524). The Examiner contends that Meier shows (Fig. 2) a surgical repair material comprising a fabric 15, a barrier layer 14 and an edge barrier 12. Applicants respectfully traverse these rejections.

As indicated above, independent claims 53 and 65 recite, *inter alia*, a prosthesis or repair fabric with an edge barrier that inhibits the formation of adhesions with tissue or organs thereto, wherein the edge barrier isolates or covers at least a portion of the edge of the fabric.

Meier is directed to a non-adherent surgical dressing including a laminated pad 13 with a cellulosic layer 14 and a resin fiber layer 15 which are joined by needled resin fibers 16 using a needling and heat fusion process. The cellulosic layer has moisture absorption properties while the resin fiber layer has a porous surface which serves as the non-adherent contact surface for the wound and which permits free flow to moisture. The pad is surrounded by an edge frame or crown 12 which stabilizes the edges of the cellulosic layer and the resin fiber layer.

As explained previously, Meier does not teach or suggest that the frame has any type of adhesion inhibiting properties. Rather, Meier discloses that frame provides freedom from fraying, loose fibers, delamination and the like by surrounding and stabilizing the edges of the

cellulosic layer and the resin fiber layer. (Col. 2, lines 51-56). Meier indicates that the frame may be fabricated from any of a variety of materials, such as an inert thermoplastic substance, which is sufficiently flexible for purposes of being applied with the pad to curved body surfaces and yet which affords sufficient rigidity to stabilize the pad and prevent delamination, etc. (Col. 2, line 67 to Col. 3, line 2).

As explained above in connection with Mulhauser and discussed with the Examiner, the adhesion resistant properties of a soft tissue repair prosthesis are affected by various factors including the surface texture and pore size of the material that forms the prosthesis or portions of the prosthesis. Thus, a prosthesis may be either resistant to the formation of adhesions or promote tissue ingrowth and adhesions depending upon the particular structural characteristics of its material. As discussed above, a prosthetic material having a surface texture or porosity of approximately 10μm or more is not adhesion resistant, but rather is susceptible to adhesions with tissue or muscle.

As explained previously, Meier provides no teaching or suggestion as to any structural characteristics of the frame that would allow one of ordinary skill in the art to determine its adhesion resistant properties. Although Meier discloses that the frame may be formed from any of various materials, Meier is silent as to the structural characteristics, such as surface texture and porosity, that affect its adhesion resistance, such that one of ordinary skill in the art would not consider the Meier frame as necessarily being resistant to tissue ingrowth and adhesions to tissue and muscle. Thus, Meier does not disclose an edge barrier that inherently inhibits the formation of adhesions with tissue and organs thereto.

In view of the foregoing, claims 53 and 65 patentably distinguish over Meier, such that the rejections under §102 should be withdrawn. Meier fails to at least disclose an adhesion resistant edge barrier as recited in each of the claims.

Claim 56 and claims 68-71 respectively depend from claims 53 and 65 and are patentable for at least the same reasons.

Rejections Under 35 U.S.C. § 103

Claims 48, 49, 51, 52, 57, 58, 63, 64, 69 and 70 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Mulhauser '525 in view of Gianturco (US 5,258,000). Applicants respectfully traverse these rejections.

Without acceding to the propriety of the combination as suggested by the Examiner, claims 48, 49, 51 and 52 depend from claim 46 and are patentable for at least the same reasons set forth above. Similarly, claims 57 and 58 depend from claim 53 and are patentable for at least the same reasons set forth above; claims 63 and 64 depend from claim 59 and are patentable for at least the same reasons set forth above; and claims 69 and 70 depend from claim 65 and are patentable for at least the same reasons set forth above. Accordingly, withdrawal of these rejections is respectfully requested.

CONCLUSION

In view of the foregoing remarks, this application should now be in condition for allowance. A notice to this effect is respectfully requested. If the Examiner believes, after this amendment, that the application is not in condition for allowance, the Examiner is requested to call the undersigned attorney at the telephone number listed below.

If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicants hereby request any necessary extension of time. If there is a fee occasioned by this response, including an extension fee, that is not covered by an enclosed check, please charge any deficiency to Deposit Account No. 23/2825.

Respectfully submitted,

By: _____
James M. Hanifin, Jr., Reg. No. 39,213
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2211
Telephone: (617) 720-3500

Date: 4/11/07
x04/19/2007x

Handbook of Biomaterials Evaluation

Scientific, Technical, and Clinical Testing of Implant Materials

Macmillan Publishing Company
NEW YORK
Collier Macmillan Canada, Inc.
TORONTO
Collier Macmillan Publishers
LONDON

Printed in the United States of America

Macmillan Publishing Company
866 Third Avenue, New York, New York 10022

Collier Macmillan Canada, Inc.

Collier Macmillan Publishers • London

Library of Congress Cataloging-in-Publication Data

Handbook of biomaterials

Includes index
1. Biomedical materials—evaluation. 2. Biomedical materials—testing. I. von Recum, Andreas. [DNLM: 1. Biocompatible Materials—standards. 2. Biomedical
QT 34 H236]
R857.M3H35 1986 610'28 86-3600
ISBN 0-02-423110-X

Printing: 1 2 3 4 5 6 7 8 Year: 6 7 8 9 0 1 2 3 4

30

The Tissue Response to Implants and Its Evaluation by Light Microscopy

Stephen C. Woodward and Thomas N. Salthouse

CELLULAR RESPONSES TO IMPLANTED MATERIALS

Overview: Inflammation and Repair

The response to a biological implant is a specialized version of inflammation and repair, the mammalian reaction to local injury. This response to local injury is nonspecific. The implant may contribute to the inflammatory response or simply may be located within a site of inflammation caused by the implantation procedure itself.[1]

The testing of a new biomaterial in man, regardless of its proposed specialized use, usually follows the gross and microscopic examination of the response to the material implanted in test animals. The extent and duration of the acute and chronic inflammatory response evoked by subcutaneous or intramuscular implants anticipates the response to them in parenchymal organs or other specialized sites.[2] Furthermore, the extent and duration of the inflammatory response are similar in rodent, dog, or rabbit to those found in primates, allowing accurate inferences to be obtained from responses in test animals as to the anticipated responses in man.[3]

The surgical process of introducing an implant itself incites an inflammatory response; hence, during the first 24 to 48 hr following implantation, the implant will be seen within a field of acute inflammation, the result of the procedure itself. During this period, the cardinal signs of acute inflammation: *redness* (the result of generalized local vascular dilatation), *heat* (the result of increased local blood flow), *swelling* (the result of increased local vascular permeability), and *pain* (the result of the local accumulation of chemical mediators or the result of increased tissue tension in a restricted space) will develop. Yet after 2–3 days, a persistent local inflammatory response to the implant, if such is present (as contrasted with the resolving, declining response to the procedure itself) can be distinguished. Because an

understanding of the source of inflammation (the procedure or the implant itself) is paramount, the events characterizing inflammation will be examined. The inflammatory response generally is reviewed in detail by Ryan and Majuo[4] and Hurley.[5]

The usual occurrence when a "nonabsorbable" material is implanted consists of transient acute inflammation (less than a week), followed by slowly developing encapsulation by fibrous connective tissue, with or without a giant cell response, requiring 1 month or longer to develop. The capsule surrounding an implant in species other than rodents is static and permanent. In rodents, by contrast, such an implant capsule may become transformed into a sarcoma after 6 months, a fact to be reckoned with in long-term testing.

Necrosis of Cells and Tissues—The Trigger of Inflammation

Necrosis, the death of cells and tissues, sets in motion a series of events aimed at leading to healing and restitution of function. Morphological changes in necrotic cells include shrinkage and intense staining of the nucleus (pyknosis), as well as fragmentation of the nucleus. Necrosis sets the inflammatory response in motion, permitting the removal by digestion of dead cells, as reviewed by Golden.[6] Persistent or intensifying acute inflammation or the accumulation of necrotic inflammatory cells or necrosis of stromal cells, as contrasted with resolution of these events, suggests that the implant is releasing chemical products that are locally toxic (Figure 30–1a).

Mast Cells and Vascular Dilatation

An initial, triggering event in inflammation is the degranulation of mast cells. Unlike granulocytes, mast cells are permanent residents of perivascular connective tissue. As described by Levy,[7] these 15 μm granule-rich cells contain heparin (an anticoagulant), histamine [a potent agent increasing vascular permeability and diapedesis (transvascular passage) of leukocytes], as well as serotonin (which is also vasoactive). Proper visualization of mast cells requires the use of metachromatic dyes; thus, mast cells are not apparent in usual histological sections. The degranulation of mast cells (Figure 30–1b) and other chemically mediated events lead to increased blood flow and vascular permeability at sites of inflammation. Increased vascular permeability is followed by the local accumulation of protein-rich edema fluid and migration of neutrophils and other inflammatory cells through the walls of venules. The blood and lymphatic vessels participate throughout the inflammatory response: The earliest events of inflammation consist of transient vascular constriction, followed by vascular dilatation, and increased blood flow.

Vascular Responses in Inflammation

Increased vascular permeability mediated by endogenous permeability factors results in increased viscosity of the blood as protein is lost to the extracellular space, with progressive stasis of blood flow and the development of edema. Local cellular necrosis developing at the same time is usually associated with damage to nearby vascular endothelium within the capillary microvasculature. Extensive and prolonged leakage of red blood cells, fibrin, and other proteins accompanied by thrombosis of the microvasculature results and is characteristic of necrotizing inflammatory responses with accompanying vascular injury. As the acute inflammatory response subsides, dilatation of the preexisting vascular bed diminishes, and the development of a new capillary bed takes place. The newly forming capillaries are part of the granulation tissue that will repair the site of injury.

Granulocytes and Acute Inflammation

At any site of inflammation, polymorphonuclear leukocytes (neutrophils) predominate over other inflammatory cells. These bone marrow-derived cells are attracted by chemoattractants released locally by damaged cells or by the implant and migrate through the walls of venules (Figure 30–1c). Granulocytes are the first defense against bacteria, being armed with peroxidases, lysozyme, and other proteolytic and hydrolytic enzymes contained within granules. These same enzymes, when released by degranulation, contribute to the degradation of such materials as catgut and silk sutures.[8]

Remove the cause for a persistent acute

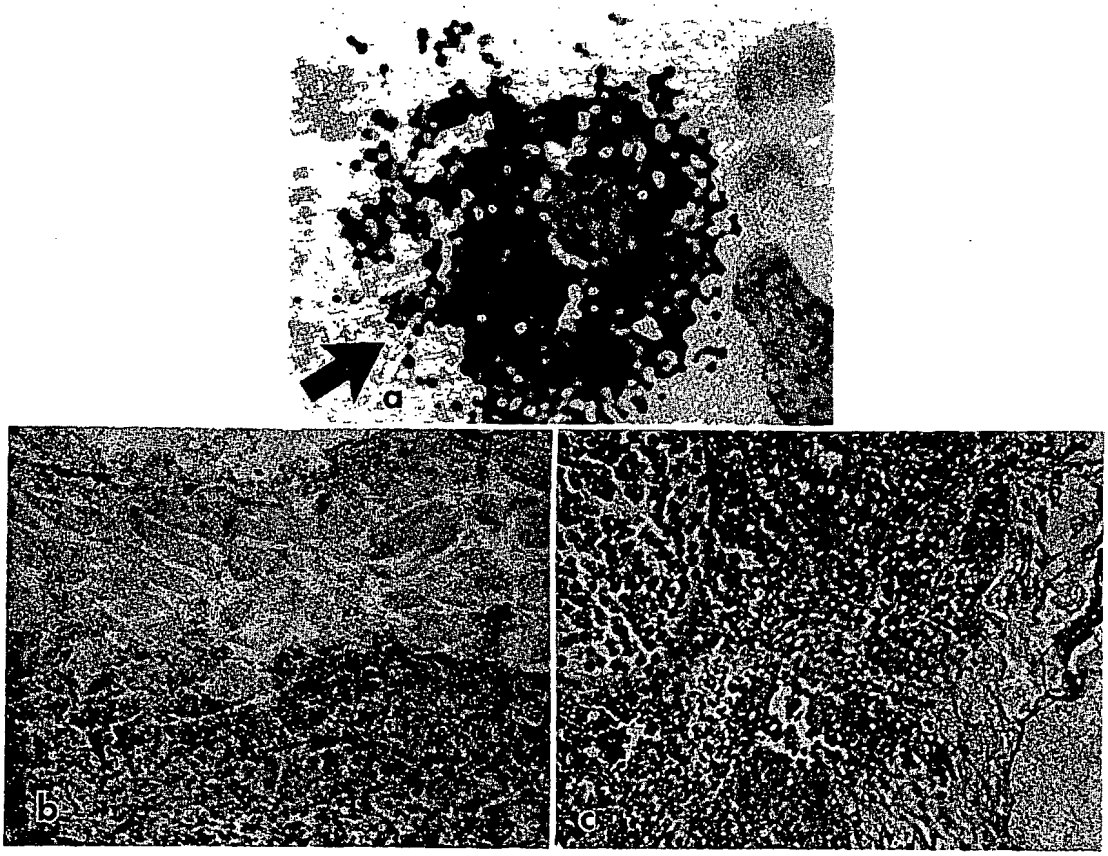

Figure 30–1 Necrosis and acute inflammation. (a) Degranulating mast cell. Mast cells are fragile, containing vascular mediators serotinin and histamine, as well as anticoagulant heparin. Their degranulation initiates the inflammatory response. Giemsa, ×500, (b) Necrosis of tissue cells is the principal trigger of the inflammatory response. Pink, necrotic muscle cells at top were chemically killed by cyanoacrylate. Inflammatory response (bottom) is adjacent to necrotic muscle. Injury is 2 weeks old. Hematoxylin and eosin. ×50, (c) Inflammatory cells are delivered to site of injury through permeable blood vessels, mostly postcapillary venules. Injury is 5 days old. Leukocytes marginate and escape through intercellular pores, migrating toward site of injury. Dilated vein on left, site of necrosis on right. Hematoxylin and eosin. ×127.

inflammation, and neutrophils disappear from an inflammatory focus after 4–6 days. Under a variety of circumstances (e.g., infection, degradation of biomaterials, or extensive necrosis of tissue due to other causes), persistent acute inflammation is found. As an example, marked neutrophil infiltration has been observed for weeks following subcutaneous deposition of methyl 2-cyanocrylate.[9] The persistence of leukocytes at an implantation site requires investigation. The usual causes include infection and biodegradation of the implant. Infection can be established as the cause by identifying microorganisms utilizing tissue Gram stain, or other histological stains for microorganisms, or with greater sensitivity by culturing the implantation site. Biodegradation (or the release of materials being leached from the implant) can be inferred when the site is found to be sterile. Biodegradation can be established as the cause of persistent acute inflammation by measuring the excretion products of isotopically tagged polymeric implants.[10] The inflammatory properties of the degradation products can be determined if these degradation products can be chemically isolated, characterized, and directly tested by implantation into animals or exposure to cell cultures.

The cytological details of granulocytes are better visualized in a Wright-stained blood film than a histological section. In a blood film, neutrophils are easily distinguished

from eosinophils (less frequently observed granulocytes participating in allergic reactions, but are rarely observed in inflammatory responses in tissues) and basophils (circulating, low-frequency cells resembling mast cells in function). In tissue sections, extravascular neutrophils initially exhibit a characteristic trilobular nucleus resembling a three-leafed clover, a feature distinguishing them from the slightly larger monocyte. After degranulation, this characteristic nuclear morphology is usually lost.

The persistence of neutrophils in tissues implies their continued accumulation there, because of their 48-hr or shorter viability extravascularly.[11] The persistent and progressive accumulation of leukocytes, whatever the cause, leads to abscess formation, with leukocyte-mediated proteolytic digestion of local tissue structures and ultimate resolution by drainage at the nearest accessible surface.

Monocytes and Macrophages—Cells of Subacute Inflammation

The morphology and functions of macrophages are reviewed by Carr[12] and Vernon-Roberts.[13] A brief summary is provided here.

Macrophages arrive at the site of an inflammatory response somewhat more slowly than do neutrophils, but their persistence and phagocytosis promote organization (the transformation of an exudate into vascularized connective tissue) and lead to repair. More robust than neutrophils in resisting the acidic conditions resulting from degranulation of neutrophils, the macrophage is the ultimate scavenger cell of the host.

The mononuclear phagocytes or macrophages, like the granulocytes, are of bone marrow origin, circulate in the blood as monocytes, and finally reside in the tissues as the tissue macrophage, also referred to as histiocyte.

In the light microscope, macrophages generally exhibit an ovoid or irregular shape, with plentiful cytoplasm and with an oval or kidney-shaped nucleus (Figure 30–2a–c). In electron microscopic preparations, projections from the cell surface are often prominent. Fingerlike projections (pseudopodia) or platelike projections (lammelapodia) are characteristic of monocytes. With enzyme histochemical preparations, macrophages demonstrate much higher hydrolase activity than either the granulocytes or fibroblasts. Such activity is not observed with either lymphocytes or plasma cells.

The enzyme activity of macrophages is contained within intracellular bodies termed lysosomes, manufactured and packaged by the Golgi zone of the cell. These enzymes play a major role in the prime function of the macrophage, enzymatic intracellular digestion following phagocytosis. Phagocytosis by this cell is generally accomplished by a process termed endocytosis, by which cellular debris or foreign material is brought into the cell. The cell membrane enfolds the particle and rejoins, internalizing the material. The vesicle containing the engulfed material generally combines with an enzyme-containing digestion vesicle, the primary lysosome, to form the secondary lysosome. The hydrolases within lysosomes will then in many cases degrade the ingested material. Other macrophages, called epithelioid cells, because of their epitheliallike appearance, are seen in hypersensitivity reactions. Epithelioid cells probably are exclusively secretory, rather than phagocytic in function.[14]

Activated macrophages (macrophages prepared for or engaging in phagocytosis) will be observed at the surface of all implanted biomaterials, but their numbers will be proportional to the toxicity of the implant material. With smooth-walled, nontoxic materials, the macrophage population is never conspicuous, and by 2 weeks macrophages will be largely replaced by fibroblasts and later by a fibrous capsule. With implants having rough or irregular surfaces, macrophages will remain active at the interface for many months. Under these circumstances, macrophages will undergo cell division, and a self-replicating population of macrophages will remain adjacent to the implant.

Lymphocytes and Plasma Cells—Cells of Chronic Inflammation

The role of plasma cells and lymphocytes is reviewed by Robbins and Cotran[2] and summarized here. Lymphocytes are round, 5–10 μm cells derived both from the bone marrow and from lymph nodes. They are characteristically present at sites of chronic

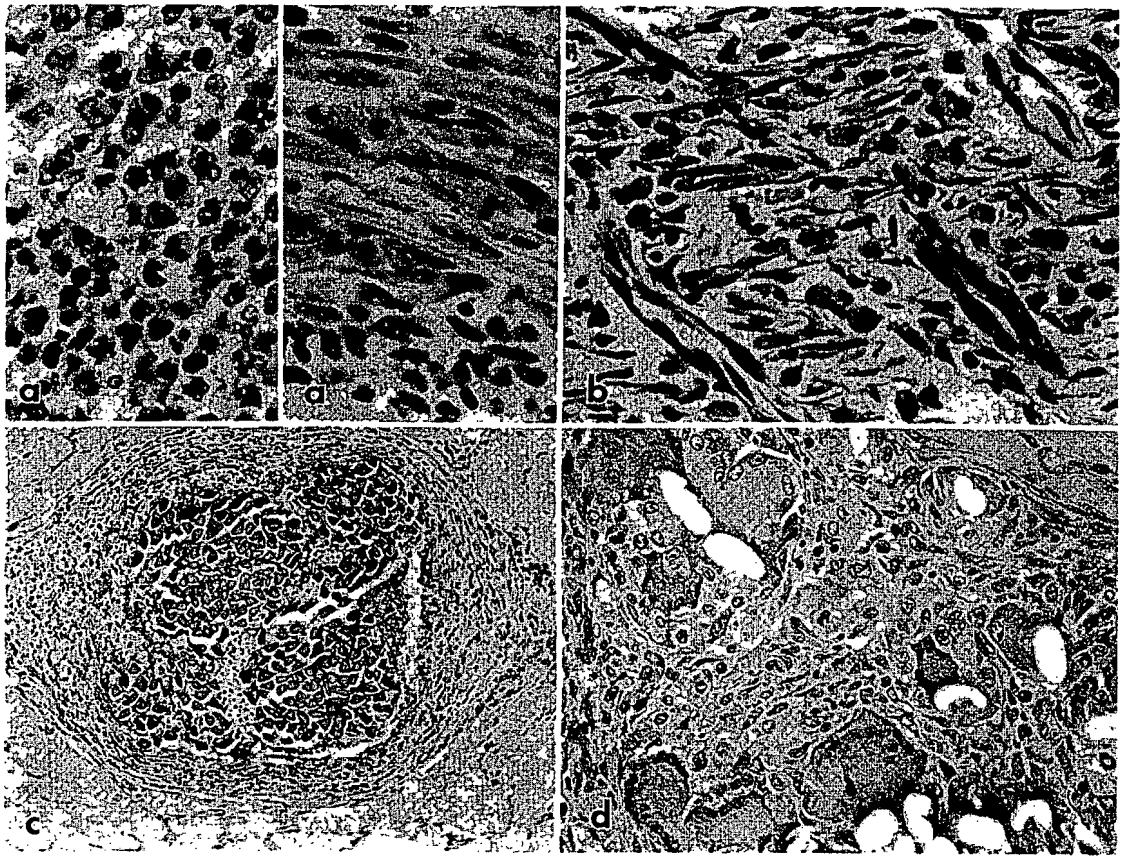

Figure 30–2 Chronic inflammation and repair. (a) Left: acute response containing neutrophils and occasional macrophages and plasma cell. Implant below, 4 days. Hematoxylin an eosin. ×16. Right: transition from acute to chronic response. Implant at bottom left. A few neutrophils remain together with macrophages and an outer population of fibroblasts in the primary stage of capsule formation. Seven days postimplant. Hematoxylin and eosin. ×160; (b) Granulation tissue at 14 days. Capillaries and fibroblasts are oriented tangentially, but in an ordered array. Mononuclear cells, plasma cells and pink collagen fibers are visible. Hematoxylin and eosin × 250 (c) Multifilament sutures produce an encapsulated foreign-body response. 7-0 silk suture within rabbit muscle at 7 days. Zone of reaction contains neutrophils, macrophages, and fibroblasts. Suture filaments are being separated by reacting cells. Hematoxylin and eosin. ×35; (d) Typical chronic granulomatous response evoked by presence of cotton fibers in rat tissue at 3 weeks. Many multinucleated giant cells surround fibers. Activated macrophages are present, represented as the smaller cells between the giant cells. Hematoxylin and eosin. × 64.

inflammation. Small numbers of lymphocytes are always present at sites of resolving injury, and large collections of lymphocytes are characteristic of immunological injury.

Plasma cells are produced from bone marrow-derived lymphocytes, and are about 20 μm in diameter. Their eccentric, cartwheellike nuclei and intensely staining cytoplasm identify them. Plasma cells are a principal source of immunoglobulins, which are secreted into sites of chronic injury. At sites of chronic inflammation, the numbers of plasma cells and lymphocytes is related to the underlying cause of the inflammation, and in conditions of chronic immunological injury, such as chronic infection or rheumatoid arthritis, these cells predominate over other inflammatory cells. Implants having antigenic properties, such as amino acid polymers, may incite a response in which plasma cells and lymphocytes are prominent. These cells are also present at sites of persistent chronic inflammation in which granulomas develop, but the role of plasma cells

and lymphocytes in chronic inflammation in the absence of immunological injury is not understood.

Giant Cells, Granulomas, and Chronic Inflammation

In addition to macrophages, plasma cells, and lymphocytes, the chronic inflammatory response surrounding nonabsorbable materials contains foreign-body giant cells. Giant cells are formed at sites of chronic inflammation from the fusion of monocytes, newly arriving from the blood, with aging macrophages.[15] Giant cells are conspicuous at sites of granulomatous inflammation in chronic infections such as tuberculosis; the granuloma consisting of discrete, circumscribed collections of macrophages and other chronic inflammatory cells. A wide variety of agents evoke granulomatous inflammation, including most microorganisms causing delayed hypersensitivity. Other materials inciting the formation of granulomas include cotton fibers (Figure 30–2d), silica, talc, beryllium, and zirconium.[16] No unique role for giant cells in chronic inflammation is evident because their phagocytic function is less developed than that of macrophages and their ability to secrete enzymes is similar to those of macrophages. Giant cells also represent the boundary between the host and many forms of implants, as will be described.

Granulomatous inflammation heals by fibrous scarring once the inciting cause either is eliminated or completely localized. A special modified form of granulomatous inflammation represents the ultimate host response to nonabsorbable or slowly absorbable implants with irregular surfaces or multiple filaments, such as braided sutures. This contrasts with the response to implants recognized by the host as smooth surfaced. When multifilament sutures or other sutures with rough surfaces are implanted, irrespective of the site, large, often highly irregular foreign-body giant cells, on occasion containing 100 or more nuclei and measuring up to 200 μm invest every surface irregularity and act as the boundary between the host and the implant. Both macrophagea and giant cells phagocytize polymer fragments and cellular debris. When the biomaterial cannot be degraded, this response is permanent, and turnover of cells is indolent. The local response just described is accompanied by the development of small blood vessels and the elaboration of collagen.[14]

Encapsulation and Organization—A Specialized Form of Wound Repair

The Development of Granulation Tissue and the Secretion of Collagen. Beginning when acute inflammation subsides, granulation tissue forms at sites of injury or tissue implantation. Granulation tissue, a transient, specialized organ of repair devoid of nerves, consists of capillaries, collagen-secreting fibroblasts, contractile fibroblasts (myofibroblasts), and chronic inflammatory cells. Granulation tissue is the site of elaboration of reparative collagen, although collagen is also slowly formed in uninjured tissue. Open wound beds consist of granulation tissue, whose myofibroblasts impart considerable contractile force to the wound bed, forces as large as 3.2×10^4 dynes/cm^2.[17] Rapid blood flow through granulation tissue provides a metabolic environment permitting fibroblasts to proliferate and elaborate collagen. The extent of granulation tissue development adjacent to prosthetic implants relates to their size and surface characteristics, as well as their degree of chemical inertness.[18] Large, rough-surfaced implants evoke extensive granulation tissue development and subsequent broad scars.

Collagen formation within granulation tissue has been examined in skin wounds. In skin wounds, collagen elaboration takes place in three phases, a lag phase (lasting a few days, during which inflammation predominates) and a log phase (lasting several weeks, during which the rapid secretion of collagen and the accretion of tensile strength parallel each other), followed by a lengthy phase of remodeling and maturation (during which collagen becomes internally and externally cross-linked and insoluable). This sequence is greatly retarded when acute inflammation persists, such as when infection supervenes or when the chemical breakdown products of a biodegradable material cause persistent necrosis of tissues.[9]

Collagen is secreted at sites of repair by fibroblasts. At least ten immunologically distinct collagens have been recognized, many

occurring only in small quantities at specialized anatomical sites. In wound repair, two principal types of collagen predominate: type I, normally found in skin and tendon, and secreted by fibroblasts, and type III, a normal constituent of the aorta, lung, and embryonal skin, and probably secreted at sites of repair by myofibroblasts. Collagen is a rigid triple helix containing glycine-rich, repeated amino acid triplets as -Gly-AA_1-AA_2, with the signal amino acids hydroxyproline and hydroxylysine in position AA_2. The structure of collagen and the location and function of many types of collagen is reviewed by Prockop *et al.*[19] The oxidation of the amino acid sequence Gly-Pro-Pro to Gly-Pro-Hypro by prolyl hydroxylase (protocollagen hydroxylase) provides a quantifying unique amino acid, hydroxyproline. Measures of hydroxyproline and the enzyme prolyl hydroxylase permit quantitation of collagen formation at repair sites.

Hydroxyproline formation is associated with formation and maturation of collagen; enzyme-mediated hydroxylysine formation is associated with the addition of carbohydrates to linked collagen.

The formation of collagen involves the cellular sequences common to proteins generally utilizing the following:

1. The ribosome: the site of α-chain synthesis and some hydroxylation
2. Extraribosomal, intracytoplasmic hydroxylation, triple helix formation, glycosilation, and secretion
3. Extracellular (partly intracellular) removal of NH_2 and COOH terminal extensions, fiber formation, cross-linking (maturation)
4. Extra- and intramolecular cross-linking, greatly increasing tensile strength and reducing solubility

Collagen is partly degraded both intracellularly and extracellularly in the process of secretion and aggregation, and secreted collagen matures by forming intra- and interfibrillar cross-linkages to produce the insoluble final product.

Light and electron microscopy are imprecise in quantitative measurement of the amount of collagen at a repair site, or in estimating the amount of collagen being elaborated, even when special staining procedures are used.[20] By contrast, the collagen content of granulation tissue can be precisely determined by measuring its hydroxyproline content.[20] A sensitive measure of the chemical toxicity of a polymeric implant is its ability to inhibit local collagen formation.[20]

Long-Term Encapsulating Responses

Inert Smooth-Surfaced Implants. Materials that are chemically nonreactive, such as monofilament nylon sutures, coupons of stainless steel, silicon disks, or Teflon rods, are surrounded by circumferentially oriented fibroblasts within 2 weeks of implantation. The fibroblasts secrete collagen oriented parallel to the implant's surface, and the ultimate response contains only a few vessels, rare lymphocytes, and monocytes and is devoid of giant cells. The zone of repair is more compact in muscle than in subcutaneous tissues and appears more compressed and cellular than in the subcutaneous space. Careful removal of such implants often reveals a tiny amount of fluid within the pocket (S. C. Woodward, unpublished observations). The interface between the host and the foreign body is permanent, and the cells and collagen participating in it turn over extremely slowly after the interfacing response is established (S. C. Woodward, unpublished observations).

Inert Implants with Irregular Surfaces

Surface irregularities as small as 10–15 μm result in the development of giant cells as the principal cellular interface between the host and nonreactive implant. The cytoplasmic processes of the giant cells adapt to surface irregularities with fidelity, anchoring to the implant. No fluid-filled pocket develops (S. C. Woodward, unpublished observations). The giant cells are surrounded by irregular, generally circumferentially oriented collagen and small blood vessels. The turnover rate of cells and collagen after this response is established is very slow.[21]

Biodegradable Implants

The response to biodegraded materials is controlled partly by the rate of degradation, but more significantly by the toxicity of the breakdown products. The corrosion products of metallic implants usually are incorpo-

rated into macrophages or giant cells and incite little other response from the host. The breakdown products of chromic catgut sutures evoke a moderately persistent inflammatory response that diminishes as the suture degrades. Polyglycolic acid evokes much less of a response than catgut because it slowly disappears.[8] Surprisingly, some materials, such as polyester and silk, generally thought of as nondegradable, appear to degrade slowly, but evoke little or no persistent inflammation.[8]

The reaction to a biodegradable material is controlled both by its surface and structure and by its degradation products.[20] Multifilament silk sutures evoke an unusual response: the suture is "exploded," its filaments being separated by infiltrating monocytes and a few neutrophils. A wide zone of fibroblasts surrounds the suture. Catgut sutures degrade similarly, except that neutrophils persist at the site. Polyglycolic acid and polylactic–polyglycolic copolymer sutures evoke a milder response than catgut, yet sites of their resorption appear active, with persistence of a mixed inflammatory response, as well as occasional giant cells and blood vessels.[8]

A still more reactive biodegradable material is methyl 2-cyanoacrylate. The degradation of this tissue adhesive is associated with a necrotizing acute inflammatory response persisting for months in which neutrophils predominate. The degradation of methyl 2-cyanoacrylate inhibits the development of collagen and thus retards repair.[9]

These examples illustrate how the local response is modified by the surface structure of the implant and its degradation products. The responses described are similar irrespective of the site of implantation.[8]

Foreign-Body Oncogenesis in Rodents

Long-term implants of nondegradable films or disks in rodents induce neoplasms peculiar to rats, mice, and hamsters. The following are well-established facts concerning this process:[22,23]

1. Any smooth surfaced nondegraded implant is capable of inducing fibrosarcomas at subcutaneous implantation sites in rats, mice, or hamsters.
2. Induction requires subcutaneous placement for 4 months or longer, a smooth, nonperforated surface, and a film size of 0.5 cm or greater.
3. The process of tumor induction is physical or geometrical, not chemical, because a wide variety of materials, including noble metals, glass, and polymeric materials, all induce sarcoma formation.
4. The neoplasms are pleomorphic fibrosarcomas that appear to arise from transitional, round, pleomorphic cells within the capsule of the implant.
5. Most foreign materials are not oncogenic in man, asbestos fibers being a notable exception.
6. No convincing association between implants in man and local or distant neoplasms have been identified, although sarcomas attending vascular grafts and mesh implants have been reported. Breast and hip prostheses have not been associated with neoplasms in man.

Despite the dichotomy between primates and rodents regarding solid-state oncogenesis, 6-month or longer implantation tests in rats, mice, and hamsters risk the accidental induction of solid-state tumors; hence, nonrodent test species, such as rabbits, should be employed to obviate the problem (for details see Chapter 17).

It is especially important in testing a new chemical formulation to avoid inadvertent induction of solid-state tumors in rodents with it. With well-studied materials widely employed as sutures or meshes in man, such as stainless steel and Teflon, the induction of neoplasms in rodents is ignored from a regulatory viewpoint, yet a new chemical formulation causing the same result would probably be subject to intense scrutiny. The subject of foreign-body carcinogenesis in rodents as it relates to health risk in man has been reviewed by Brand[22] and Woodward.[23]

IMPLANT PROPERTIES AFFECTING TISSUE RESPONSE

The variables that significantly influence the local histological response to an implant include surface characteristics, size, chemical composition, and location of implantation. Each of these requires careful control, especially when attempting to compare the

reactivity of an uninvestigated material under development with those already in use.

Surface Characteristics

Surface characteristics include both geometrical configuration and surface irregularities. By comparing the responses to extruded 1-mm diameter cylinders with those to pentagonal and triangular rods of the same surface area composed of various nonabsorbable polymers, including polyvinyl chloride, Salthouse and Matlaga showed that the circular configuration evoked a narrower zone of fibrosis than that seen surrounding the triangular or pentagonal implants, particularly at the apices of their angulations.[18] Furthermore, lysosomal enzyme activity, as measured by acid phosphatase content of the capsule surrounding a circular implant, at 14 days was only half that found adjacent to a triangular implant. Thus, to control for geometrical effects, materials under development and being evaluated for histocompatibility should be implanted as cylinders.

Furthermore, surface characteristics affect the response. Surface irregularities promote the development of a foreign-body giant cell response as contrasted with the circumferentially oriented collagen comprising the wall of a fluid-filled pocket, which is the response to nonreactive, smooth-surfaced materials (Figure 30–3a, b, c,). The effects produced by surface irregularities were examined by abrading Teflon rods and comparing the effects produced by abrasion on the histochemistry of the surrounding capsule. Salthouse and Matlaga[18] observed that the capsules surrounding abraded rods, as com-

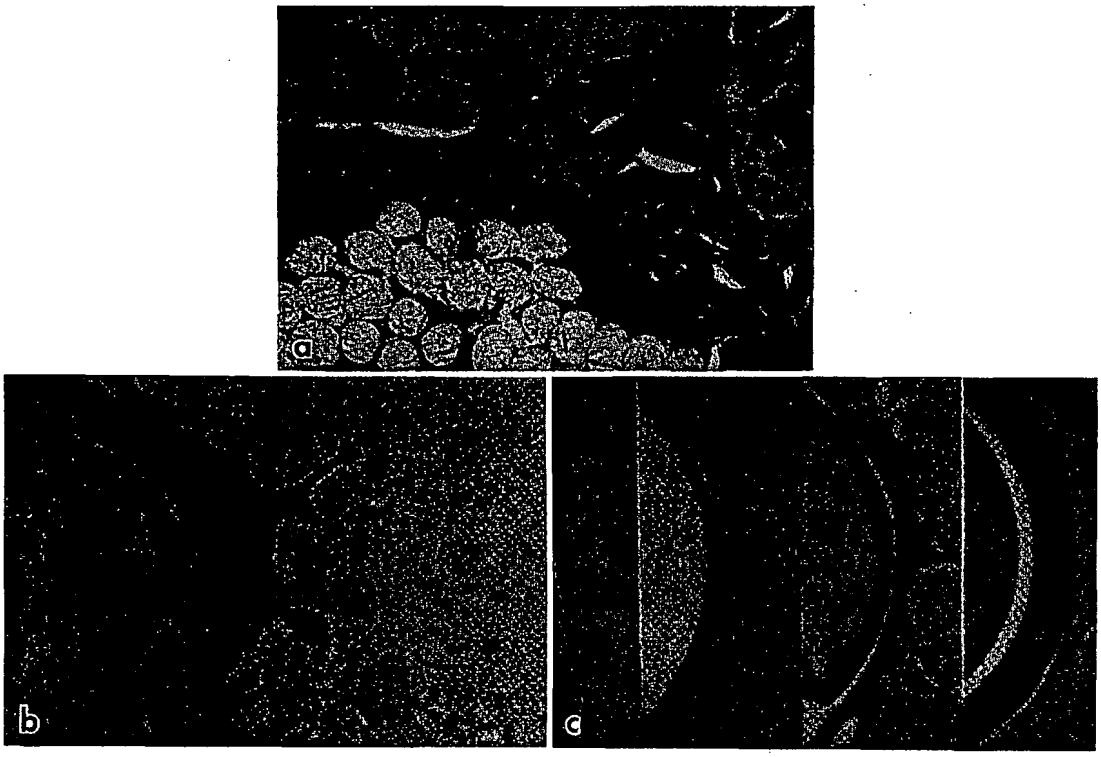

Figure 30–3 Encapsulating responses. (a) Tissue reaction to biodegradable copolymer suture at 2 weeks. Macrophages and giant cell adjacent to implant with fibroblasts and developing collagen fibers (red) at periphery, are stained yellow and nuclei black. Stained by Van Gieson picrofuchsin after Weigert's iron hematoxylin. × 55. (b) Foreign-body giant cells may contain several hundred nuclei, as well as attenuated cytoplasmic processes. This giant cell occupies a portion of the edge of a 4-month deposit of hexyl α-cyanoacrylate, a bland, nondegraded material of irregular contour. Hematoxylin and eosin. × 145; (c) Response to monofilament nylon suture at 1 week, 3 weeks, 3 months, and 6 months in rabbit muscle. In this methacrylate-embedded preparation muscle stains yellow, collagen pink. The encapsulating collagen follows the contour of the suture. Inflammation is absent. Stain as in (a) × 230.

TABLE 30–1

Effects of Structure on the Response to Implants

CONFIGURATION	ULTIMATE RESPONSE
Circular, smooth*	Circumferential fibroblasts and collagen, minimal response
Angular, smooth	Collagen and oriented parallel to surface, chronic inflammation at angularities
Rough, irregular	Foreign-body giant cell response, chronic inflammation, and persistent vascularization
Powders* ($\lambda\epsilon\sigma\sigma\ \tau\eta\alpha\nu_{250}\ \mu m$)	Foreign-body response with phagocytosis of powder by macrophages and giant cells; granulomatous inflammation
Porous (polyvinyl alcohol sponge, porous ceramics)	Infiltration of granulation tissue ultimately producing dense fibrous entrapment

* Both responses can be elicited by the same material, such as PTFE (Teflon), depending on the physical structure.

pared with smooth-surfaced ones, showed persistently increased levels of acid phosphatase (lysosomal activity), glucose-6-phosphate dehydrogenase (glycolysis), and succinic dehydrogenase (oxidation–reduction) for 90 days. These enzyme activities were localized to the increased populations of macrophages surrounding the abraded rods. Encapsulation was retarded by abrasion. These results strongly suggest, as a standard condition, that a newly tested material have as smooth a surface as possible. Obviously many materials are fabricated as meshes, weaves, or other complex geometries, with varying degrees of surface irregularities. The effects of such fabrication can be identified, if the response to a standard, smooth-surfaced cylinder has been determined. The effects of the physical structure of the implant on the host response are summarized in Table 30–1.

Size of Implant

Generally, small implants give more uniform responses than larger ones, in part because they tend to migrate less and their movement is less traumatic to surrounding tissues than movement by a larger implant. If possible, nonabsorbable materials should be fabricated as cylinders 1–4 mm in diameter and 10 mm or less in length. Many such implants can be placed in the same animal and removed sequentially, if necessary. Flat coupons of metal measuring about 10 mm square and 1–2 mm thick are often employed to examine the reactivity and corrosion of the material.

Chemical Composition

In addition to the toxic effects of impurities present on the surface of the implant or plasticizers that may be leached from it, other chemical features related to biodegradation strongly affect the histological response to a degradable material. In general, active enzymatic breakdown of a biodegradable is associated with persistent inflammation, such as the mononuclear response to degrading catgut[8] or the persistent, intense infiltrate of neutrophils attending the degradation of methyl 2-cyanoacrylate.[9] In addition, biodegradable polymers tend to undergo loss of molecular weight with resultant change in physical characteristics, such as increasing brittleness resulting in fracturing and powder formation. This may result in a previously quiescent capsule being replaced by one of foreign-body response with phagocytosis of the powder, as was observed in the ultimate degradation of poly(ϵ-caprolactone).[24] The degree of reactivity of various materials is given in Table 30–2. This table also suggests suitable positive controls (highly reactive materials) and nonreactive, negative controls.

Location of Implant

The initial testing of polymeric material is usually carried out in the subcutaneous space or in the deep musculature. The response at these sites is similar and is generally predictive of responses elsewhere. Cardiovascular materials generally elicit the same local response in a vascular anastomotic site as within a muscle, although repair and organization is generally slower and more incomplete than in muscle, and thrombogenic effects may slow the organizational response further.[25] Materials implanted in a parenchymal organ, such as the kidney, will elicit the same basic response as in subcutaneous tissue and muscle. The inflammation and scarring around the implant will result in destruction of parenchymal elements and their replacement with connective tissue.

TABLE 30–2

Reactions Produced by Implants

	IMPLANTATION/SITE	RESPONSE
Nonreactive, nonabsorbable[6]		
Monofilament nylon, PTFE (Teflon) or polypropylene sutures	Muscle or subcutaneous	Narrow, fibrous capsule
Silicone elastomers[30]	Subcutaneous	Fibrous capsule
Nonreactive, biodegradable[31]		
Polyglycolic acid and polyglycolic–polylactic copolymer monofilament sutures	Muscle or subcutaneous	Minimal chronic inflammation, no capsule
Intermediate		
Catgut sutures[6]	Muscle	Persistent mononuclear and giant cell response
Stainless steel monofilament	Muscle	Mononuclear response, first 2–4 weeks
Strongly reactive, biodegradable		
Methyl 1 2-cyanoacrylate[9]	Subcutaneous, muscle	Persistent acute inflammation and necrosis
Resins containing organometallics, cadmium, or chromium[32]	Subcutaneous	Persistent acute inflammation

SUGGESTIONS FOR EXPERIMENTAL DESIGN

In examining a new chemical formulation, the following questions should be addressed:

1. Is the material biodegradable? This question can usually be resolved by observation for persistent acute inflammation at 2 weeks or the persistence of numerous macrophages beyond 3–4 weeks at subcutaneous or intramuscular implantation sites because persistent inflammation often accompanies biodegradation. Thus, observations at 1, 2, 4, and 8 weeks generally will suffice to detect early biodegradation or continued reactivity, although observations for as long as 2 years may be required to detect slow breakdown, and physical measures of the integrity and weights of recovered implants, accompanied by studies of excretion on isotopically tagged polymers, ultimately may be required for resolution of the issue of biodegradation.[10]

2. If so, does the degradation process evoke an acute inflammatory response? Is the time sequence of the encapsulating response prolonged, suggesting continuing reactivity or biodegradation? Measurement of the zone of acute or chronic inflammation around an implant is of value in quantifying reactivity and comparing reactivity to that of a negative control; similarly, measuring the width of acid phosphatase reactivity can also serve this purpose.[18] Prolonged inflammation, and slow development of a capsule suggests a toxic local response. Specific methods for this evaluation are given in paragraph 3, below.

3. If the material does not degrade, is a smooth-surfaced cylinder encapsulated by a narrow zone of fibrous repair similar to that observed with an appropriate negative control (see Table 30–2 for examples)? The following plan is suggested for the evaluation:

Various approaches have been suggested to increase objectivity and to evolve a scoring or rating system for the tissue response or reaction to implants. An earlier suggestion by Sewell *et al.*[26] and later ones by Gourlay,[27] Salthouse,[28] and Black[29] are useful in ranking the biocompatibility of samples. It is essential that samples to be compared are of identical shape and surface.[18] A selection of methods is given below.

Sample Implantation

Implantation in rat gluteal muscle or rabbit paravertebral muscle is suggested. Samples must be identical in shape and surface. For polymers 10–15 mm in length, 0.5 mm diameter can be implanted using a wide bore needle and trochar. A low reactive control, such as polypropylene, should be included.

Implantation periods of 7, 21, 42, 90, and 180 days will encompass both acute and chronic inflammatory responses. The number of samples and animals should be sufficient for statistical requirements. Adequate controls should be included. Implantation under aseptic conditions is essential. Histological cross sections by either the paraffin or methacrylate procedures should be prepared and stained by hematoxylin and eosin (see Chapter 29).

RATING SYSTEM FOR 0.5 MM DIAMETER SAMPLES

Samples with greater or lesser diameters than 0.5 mm require modification.

Grades for Reaction Thickness

Grade 1	0–50 μm zone diameter
2	50–100 μm zone diameter
3	100–500 μm zone diameter
4	500–1000 μm zone diameter
5	1000–2000 μm zone diameter

Cellular Response Grade

Grade 0 to 5 depending on concentration of cells in reaction zone area (cell density).

Weighting Factors

These can be assigned based on the reaction zone characteristics, for example, see tabulation below.

Reaction zone size	5
Cell density	3
Cell type: Neutrophils	5
Giant cells	2
Macrophages	1
Fibroblasts	1
Lymphocytes	1

To continue the example, the final rating might be as follows:

Reaction zone grade 3 × factor 5	15
Cellular response grade 2 × factor 3	6
Cell types Neutrophils 5 × factor 5	25
Macrophages 1 × factor 5	5
Rating:	51

Such a numerical rating can be arbitrarily expressed as follows:

No reaction	0
Minimal	1–10
Slight	11–25
Moderate	26–40
Marked	41–60
Extreme	60 +

Such a system can be modified in several ways to suit the other sizes and types of implants or requirements.

The diameter and area of tissue reaction zones can be measured by either point counting eyepiece or by image analysis systems (Chapter 33).

Simple Numerical Quantitation of the Cellular Response

Usually with the higher cellular response around an implant, it can be assumed the greater the histotoxicity of the implant material. If sections are treated by a stain having approximately stoichiometric staining for inflammatory cells, the cell population, both in area and concentration, can be measured photometrically for concentration and by image analysis for area size. A gallocyanin nuclear stain is suggested for this application.[28]

STAIN	
Gallocyanin	.3 gm
Chrome Alum $CrK(SO_4)_2 \cdot 12H_2O$	10.0 gm
Distilled water	200 ml

Dissolve and boil 15 min, cool, filter, and store in refrigerator. Stain sections (of equivalent thickness) at room temperature for 12 hr. Rinse in distilled water for 30 sec, dehydrate in two changes of ethanol, clear in xylene, and mount. Sections can be used for photometric or image analysis as described in Chapter 33.

It will be apparent to the reader that the above, rather simple, approaches to quantitation and objectivity in histological evaluation can be modified to yield more sophisticated data by computer programming. In addition,

they are applicable to biodegradable as well as less reactive materials.

4. Does the material inhibit collagen elaboration? The elaboration of collagen is fundamental to effective wound repair, and during the early phases of this process, increasing tensile strength parallels increasing collagen content of the wound.[20] The inhibition of collagen elaboration can be quantitated by incorporating a powder of a biomaterial into polyvinyl alcohol–formal sponges that are then placed subcutaneously in the rat, removed at 7 and 14 days, and compared with controls with respect to wet weight (inflammation), dry weight (connective tissue content), and hydroxyproline content, an identifying imimo acid of collagen.[20] Histological sections of sponges can be examined for their degree of persistent inflammation and organization (as defined previously). Utilizing this procedure, the author has quantified the histotoxicity of several microscopically inflammatory cyanacrylates,[9] by measuring their local inhibition of the accumulation of hydroxyproline. Furthermore, polyglycolic acid (Figure 30–4) and poly(ϵ-caprolactone)[24] powders do not inhibit collagen elaboration by this test and are noninflammatory. Other biochemical measures of collagen elaboration, such as the accumulation of prolylhydroxylase, or of the presence of macrophage enzymes, such as acid phosphatase, can be obtained also from sponge implants as illustrated in Figure 30–4. These quantitative measures of inflammation and repair often provide more sensitive indicators of reactivity than does light microscopy, in which the evaluation of the density and amount of collagen is at best only semiquantitative.

LIMITATIONS OF LIGHT MICROSCOPY

Although light microscopy is the most commonly employed method to evaluate host–biomaterial interactions, it has several significant limitations that should be recognized:

1. The estimation of collagen production at an implant site is at best semiquantitative; additional chemical determinations are required for quantification.

2. Intracellular sites of degradation cannot be visualized when particles undergoing degradation are less than 2–4 μm in diameter; electron microscopy is required for this determination.[24]

3. Routine hematoxylin–eosin stains do not allow for quantitative analysis of the chronic inflammatory response adjacent to an implant; they should usually be accompanied by acid phosphatase and other enzyme histochemical techniques.

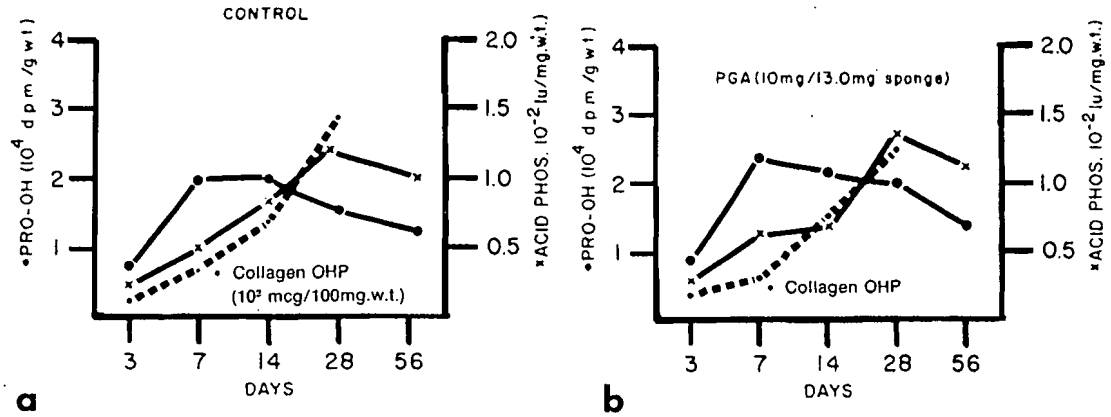

Figure 30–4 Biochemical measures of inflammation and repair. (a) The time relationships of the resolution of the inflammatory response and the subsequent elaboration of collagen, the most significant aspect of repair, can be monitored in polyvinyl alcohol–formal sponge implants. Proline hydroxylase measures cellular mobilization for collagen formation, hydroxyproline content is a measure of collagen content, and acid phosphatase content reflects continuing accumulation and degranulation of macrophages; (b) Polyglycolic acid powder incorporated into sponges evokes minimal inflammation. Collagen formation is not inhibited by it as measured by proline hydroxylase or hydroxylase content. Acid phosphatase concentration, reflecting macrophage activity, is only marginally increased at 7–10 days.

4. A false impression that bioabsorption is occurring can result from dissolution of a polymeric material in the solvents used in preparing histological sections. A clue to this would be an organizational response surrounding an empty space. Observing this, the solubility of the biomaterial in tissue-processing solutions should be assessed.

REFERENCES

1. Robbins, S. L., and Cotran, R. S.: *Pathologic Basis of Disease*, 2nd ed., Saunders, Philadelphia, 1979, pp. 94–95.
2. Robbins, S. L., and Cotran, R. S.: *Pathologic Basis of Disease*, 2nd ed., Saunders, Philadelphia, 1979, pp. 56–57.
3. Anderson, W. A. D.: *Pathology*, 6th ed., Mosby, St. Louis, 1971, p. 16.
4. Ryan, G. B., and Majuo, G.: "Acute Inflammation, A Review," *Am. J. Pathol.*, **86**:185–276, 1977.
5. Hurley, J. V.: *Acute Inflammation*, 2nd ed. Churchill Livingstone, Edinburgh, 1983, pp. 1–7.
6. Golden, A.: *Pathology, Understanding Human Disease*, Williams and Wilkins, Baltimore, 1982, pp. 3–41.
7. Levy, D.: "Histamine and Serotonin," in Weisman, G. (Ed.), *Mediators of Inflammation*, Plenum, New York, 1974, pp. 141–161.
8. Salthouse, T. N.: "Tissue Response to Sutures," in Rubin, L. R. (Ed.), *Biomaterials in Reconstructive Surgery*, Mosby, St. Louis, 1983, pp. 131–142.
9. Woodward, S. C., Herrmann, J. B., Cameron, J. L., et al.: "The Effect of Cyanoacrylate Tissue Adhesives upon Granulation Tissue Formation in Ivalon Sponge Implants in the Rat," *Surgery*, **59**:559–565, 1966.
10. Cameron, J. L., Woodward, S. C., Pulaski, E. J., et al.: "The degradation of Cyanoacrylate Tissue Adhesive," *Surgery*, **58**:424–430, 1965.
11. Janoff, A.: "Mediators of Tissue Damage in Human Polymorpho-Nuclear Neutrophils," *Ser. Haematol.*, 3:96–130, 1970.
12. Carr, I.: *The Macrophage*, Academic Press, New York, 1972, pp. 1–60.
13. Vernon-Roberts, B.: *The Macrophage*, Cambridge University Press, Cambridge, 1972.
14. Hurley, J. V.: *Acute Inflammation*, 2nd ed., Churchill Livingstone, Edinburgh, 1983, p. 142–143.
15. Chambers, T. J.: "Multinucleate Giant Cells," *J. Pathol.*, **126**:125–148, 1978.
16. Adams, D. O.: "The Granulomatous Inflammatory Response: A Review," *Am. J. Pathol.*, **86**:164–191, 1976.
17. Guber, S., and Rudolph, R.: "The Myofibroblast," *Surg. Gyn. Obst.*, **146**:641–655, 1978.
18. Salthouse, T. N., and Matlaga, B. F.: "Some Cellular Effects Related to Implant Shape and Surface," in Rubin, L. R. (Ed.) *Biomaterials in Reconstructive Surgery*, Mosby, St. Louis, 1983, pp. 40–45.
19. Prockop, D. J., Kivirikko, K. I., Tuderman, L., et al.: "The Biosynthesis of Collagen and Its Disorders," *N. Engl. J. Med.*, **301**:13–23, 77–85, 1979.
20. Woodward, S. C.: "Physiological and Biochemical Evaluation of Implanted Polymers," *Ann. N.Y. Acad. Sci.*, **146**:225–250, 1966.
21. Robbins, S. L., and Cotran, R. S.: *Pathologic Basis of Disease*, 2nd ed., Saunders, Philadelphia, 1979, pp. 84–86.
22. Brand, K. G.: "Human Foreign-Body Carcinogenesis in the Light of Animal Experiments and Assessment of Cancer Risk at Implant Sites," in Rubin, L. R. (Ed.), *Biomaterials in Reconstructive Surgery*, Mosby, St. Louis, 1983, pp. 36–39.
23. Woodward, S. C.: "How to Relate Observations of Foreign-Body Oncogenesis in Experimental Animals to Human Health Risk," in Rubin, L. R. (Ed.), *Biomaterials in Reconstructive Surgery*. Mosby, St. Louis, 1983, pp. 17–26.
24. Woodward, S. C., Brewer, P. S., Moatamed, F., et al.: "The Intracellular Degredation of Poly(ε-caprolactone), *J. Biomed. Mater. Res.*, in press.
25. Wesolowski, S. A.: *Evaluation of Tissue and Prosthetic Vascular Grafts*, Thomas, Springfield, Illinois, 1962, pp. 1–20.
26. Sewell, W. R., Wiland, J., and Craver, B. N.: "New Method of Comparing Sutures of Ovine Catgut with Sutures of Bovine Catgut in 3 Species," *Surg. Gyn. Obst.*, **100**:483, 1955.
27. Gourlay, S. J., Rice, R. M., Hegyeli, A. F., et al.: "Biocompatibility Testing of Polymers: In Vivo Implantation Studies," *J. Biomed. Mater. Res.*, **12**:219, 1978.
28. Salthouse, T. N.: *Biocompatibility in Clinical Practice*, Vol. I, Williams, D. F., (Ed.), CRC Press. Boca Raton, Florida, 1982, pp. 18 and 24.
29. Black, J.: *Biological Performance of Materials*, Marcel Dekker, New York, 1981.
30. Frisch, E. E.: "Technology of Silicones in Biomedical Applications," in Rubin, L. R. (Ed.), *Biomaterials in Reconstructive Surgery*, Mosby, St. Louis, 1983, pp. 73–90.
31. Miller, R. A., Brady, J. M., and Cutright, D. E.: "Degradation Rates of Oral resorbable Implants (Polylactides and Polyglycolates): Rate Modification with Changes in PLA/PGA Copolymer Ratios," *J. Biomed. Mater. Res.*, **11**:711–719, 1977.
32. Blais, P.: "Industrial Polymers as Implants: Their Value and Their Limitations," in Rubin, L. R. (Ed.), *Biomaterials in Reconstructive Surgery*, Mosby, St. Louis, 1983, pp. 62–72.

ADDITIONAL READING

Cellular Events and Inflammation

Adams, D. O.: "The Granulomatour Inflammatory Response: A Review," *Am. J. Pathol.*, **86**:164–191, 1976.

Anderson, W. A. D., and Kissane, J. M.: *Pathology*, 7th ed., Mosby, St. Louis, 1977, Chap. 2, Inflammation; Chap. 6, Physical Agents in Causation of Injury and Disease; Chap. 7, Drug and Chemical Injury—Environmental Pathology.

Chambers, T. J.: "Multinucleate Giant Cells," *J. Pathol.* **126**:125–148, 1978.

Hurley, J. V.: *Acute Inflammation*, 2nd ed., Churchill Livingstone, Edinburgh, 1983.

Murphy, P.: *The Neutrophil*, Plenum, New York, 1976.

Vernon-Roberts, B.: *The Macrophage*, Cambridge University Press, Cambridge, 1972.

Weissman, G.: *Mediators of Inflammation*, Plenum, New York, 1974.

Weissman, G.: *The Cell Biology of Inflammation*, Elsevier, New York, 1980.

Wound Repair and Biomaterials

Kulonen, E., and Pikkarainen, J. (Eds.): *Biology of Fibroblast*, Academic Press, London, 1973.

Peacock, E. E., and vanWinkle, W.: *Wound Repair*, Saunders, Philadelphia, 1976.

Prockop, D. J.: "The Biosynthesis of Collagen and Its Disorders," *N. Eng. J. Med.*, **301**:13–77, 1979.

In Vivo Evaluation of Biomaterials

Black, J.: *Biological Performance of Materials*, Marcel Dekker, New York, 1981.

Rubin, L. R.: "The Normal body Defenses against Foreign Implants," *Biomaterials in Reconstructive Surgery*, Chap. 1, Mosby, St. Louis, 1983.

Williams, D. F.: *Biocompatibility in Clinical Practice*, Vols. I and II, CRC Press, Boca Raton, Florida, 1982.

Williams, D. F.: *Techniques for Examination of Tissues in Implant Retrieval Analysis*, NBS Special Publ. 601, National Bureau of Standards, Washington, D.C., 1982. pp. 323–338.

Solid State Carcinogenesis

Rubin, L. R.: *Biomaterials in Reconstructive Surgery*, Mosby, St. Louis, 1983. Chap. 2, How to Relate Observations of Foreign-Body Oncogenesis in Experimental Animals to Human Health risk; Chap. 5, Some Cellular Effects Related to Implant Shape and Surface.

FERTILITY AND STERILITY
Vol. 49, No. 6, June 1988
Printed in U.S.A.

Reduction of postoperative pelvic adhesions in the rabbit with Gore-Tex* surgical membrane

Stephen P. Boyers, M.D.†
Michael P. Diamond, M.D.‡
Alan H. DeCherney, M.D.

Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Connecticut

Gore-Tex (W. L. Gore and Associates, Inc., Flagstaff, AZ) surgical membrane (SM), a nonreactive expanded polytetrafluoroethylene (PTFE), was used in 24 mature New Zealand rabbits (2200 to 3000 gm) to cover 2-cm^2 ischemic defects in the pelvic sidewall peritoneum to reduce adhesion formation in a rabbit pelvic sidewall/uterine horn injury model. SM was randomly assigned to cover one defect; the opposite defect remained uncovered, each animal serving as its own control. Rabbits were sacrificed 3 weeks later, and adhesions between uterine horn and pelvic sidewall or SM were scored for extent (0 to 4), type (0 to 4), and tenacity (0 to 3). Injury sites were removed en bloc for histologic study. The mean (± standard deviation [SD]) adhesion score for SM-covered lesions (4.3 ± 1.8) was significantly lower than for controls (9.1 ± 2.5) ($P < 0.001$; Wilcoxon Signed Rank test). By histology, none of 24 SM-covered lesions demonstrated adhesions to the membrane itself, whereas 19 of the 24 control lesions showed dense adhesions to the injury site ($P < 0.001$; chi-square test). By both gross and microscopic assessment, SM was nonadherent to the underlying sidewall defect in 100% of cases. In conclusion, Gore-Tex surgical membrane is an effective barrier for reducing primary adhesions in this pelvic injury model and offers promise for adhesion reduction in human pelvic surgery. Fertil Steril 49:1066, 1988

Peritoneal injury provides a nidus for adhesion formation. In the human, defects in the normal pelvic peritoneum frequently result from surgery for endometriosis or from lysis of pre-existing adhesions, particularly between the ovary and the pelvic sidewall. Adhesions commonly occur between the injured pelvic sidewall and adjacent ovary, tube, or bowel.[1–5] A variety of techniques has been employed to close or cover peritoneal defects or otherwise reduce adhesions but none are entirely successful. Large peritoneal injuries are particularly difficult to manage. Peritoneal closure under tension is associated with tissue ischemia and necrosis, which may stimulate rather than prevent adhesion formation.[6–8] Free grafts of peritoneum or omentum have been used, but these grafts also become ischemic and have not proved to be an effective barrier to adhesion formation.

An artificial membrane that could act as a barrier between injury sites without itself provoking adhesions might be more effective than free grafts since such a membrane would not be subject to necrosis and could be tailored to cover a variety of injury sites. Gore-Tex (W. L. Gore & Associates, Inc., Flagstaff, AZ) surgical membrane (SM) is manufactured as a thin sheet of expanded polytetrafluoroethylene (PTFE) and has been used successfully as a pericardial membrane substitute for more than 10 years.[9] In contrast to Gore-Tex vas-

Received July 20, 1987; revised and accepted February 26, 1988.

* Supported by W. L. Gore & Associates, Inc., Flagstaff, Arizona, manufacturer of Gore-Tex.

† Reprint requests: Stephen P. Boyers, M.D., Department of Obstetrics and Gynecology, Yale University School of Medicine, 333 Cedar Street, P. O. Box 3333, New Haven, Connecticut 06510-8063.

‡ Mellon Fellow in the Reproductive Sciences.

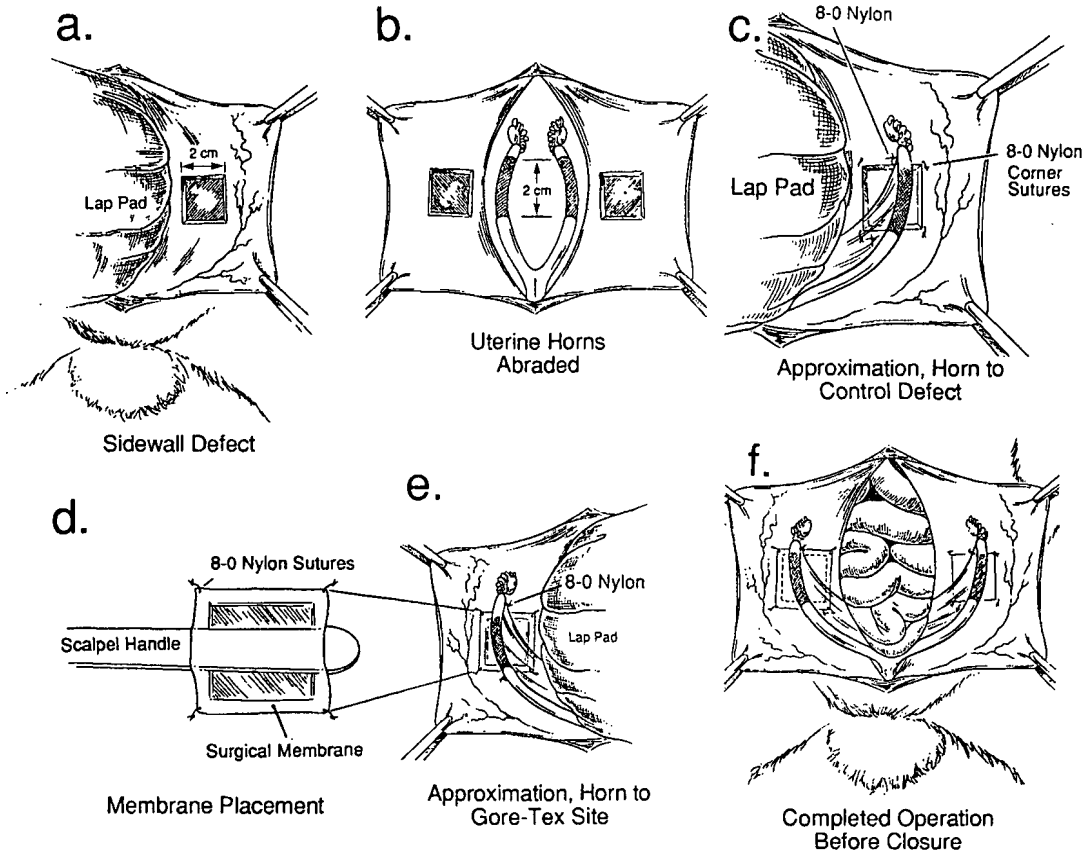

Figure 1 (a–f) Schematic of the rabbit pelvic sidewall/uterine horn injury model.

cular graft and Gore-Tex cardiovascular patch, which are designed to encourage cellular penetration and tissue adhesion, surgical membrane has an extremely small pore size, which discourages tissue attachment. These characteristics make surgical membrane a potentially useful barrier for reducing adhesions in reproductive surgery.

The purpose of this study is to evaluate the usefulness of SM as a barrier to reduce primary adhesions in a rabbit pelvic injury model.

MATERIALS AND METHODS

Twenty-four sexually mature female New Zealand rabbits weighing between 2200 and 3000 gm were operated through a midline lower abdominal incision in a sterile field under Rompum-ketamine anesthesia. Rabbits were Q-fever free and were maintained on rabbit chow and water ad libitum. Figure 1 outlines the surgical model. Each animal served as its own control. Identical 2 × 2 cm peritoneal defects were created on the left and right pelvic sidewalls by sharply excising a peritoneal patch. Underlying muscle was systematically cauterized by bipolar microcautery forceps applied equally to the left and right defects to ensure an ischemic injury. The distal 2 cm of each uterine horn was abraded with an equal number of scrapes from a scalpel blade, producing punctate bleeding. After all lesions had been completed, surgical membrane was randomly assigned to cover either the left or right sidewall defect. A square of surgical membrane was tailored to completely cover the defect, overlapping its edges by 2 to 4 mm, and was tacked in place at each corner with a single suture of 8–0 Ethilon (Ethicon, Somerville, NJ). The control lesion remained uncovered, but 8–0 Ethilon sutures were placed at the four corners to match the opposite side. To encourage adhesion formation, the ipsilateral uterine horn was suspended across the sidewall defect and was held by an 8–0 Ethilon suture placed 3 to 5 mm on either side of the injury site. The abdominal incision was closed by approximating the musculoperitoneal and cutaneous layers with continuous 3–0 Dexon sutures (Davis and Geck, Pearl River, NY). Postoperatively, all rabbits received penicillin 300,000 units intramuscularly every day for 3 days.

Table 1 Criteria for Scoring Adhesions

	Description	Score
Extent	No sidewall involvement	0
	≤25% sidewall involvement	1
	≤50% sidewall involvement	2
	≤75% sidewall involvement	3
	>75% sidewall involvement	4
Type	None	0
	Filmy, transparent, avascular	1
	Opaque, translucent, avascular	2
	Opaque, capillaries present	3
	Opaque, larger vessels present	4
Tenacity	None	0
	Adhesions essentially fell apart	1
	Adhesions lysed with traction	2
	Adhesions require sharp dissection	3
Maximum total score =		11

Three weeks after initial surgery, all animals were reoperated to assess adhesion formation. Adhesions were scored for extent, type, and tenacity, as outlined in Table 1. Total scores ranged from 0 to 11. Animals were sacrificed by pentobarbital overdose, and sidewall injury sites and ipsilateral uterine horns were removed en bloc and fixed in 10% neutral buffered formalin for histologic study. Thin sections were stained with hematoxylin and eosin (H&E), Milligan's trichrome, and fibrin stains.

Statistical analysis was performed by chi-square and Wilcoxon Signed Rank tests. A P value ≤ 0.05 was accepted as statistically significant. All data are expressed as mean ± standard deviation (SD).

RESULTS

Table 2 lists adhesion scores (mean ± SD) for control and Gore-Tex (SM) covered lesions. The mean scores for extent, type, and tenacity of adhesions were each significantly lower for Gore-Tex covered lesions ($P < 0.001$; Wilcoxon Signed Rank test). The total adhesion score for Gore-Tex covered lesions (4.3 ± 1.8) was also significantly lower than the total score for controls (9.1 ± 2.5) ($P < 0.001$).

Table 2 also compares total adhesion scores with histologic findings for control and Gore-Tex covered lesions. Light microscopy was used to distinguish true adhesions either to the uncovered sidewall defect or to SM from apparent adhesions that were in fact to suture sites. At histology, 19 of the 24 control lesions were confirmed to have dense adhesions between the ipsilateral uterine horn and the sidewall defect. None of 24 Gore-Tex covered lesions showed adhesions to the surgical membrane itself. Rather, the adhesions on the SM side were to suture sites or to parts of the sidewall defect that had not been effectively protected because the surgical membrane, tacked only at the four corners, buckled between sutures and allowed adhesions access to small areas of the lesion. All SM patches were nonadherent to the underlying sidewall defect by both gross and microscopic assessment. The defect itself on the Gore-Tex protected side appeared grossly to have reperitonealized, and this impression was confirmed histologically.

DISCUSSION

The role of peritoneal injury in the genesis of adhesions remains controversial. In animal models, clean, nonischemic peritoneal defects heal rapidly by re-epithelialization without adhesions.[10] While closure of such lesions in human reproductive surgery has been advocated by some,[11] these clean defects are probably better left alone, and heroic measures like mechanical barriers to cover these lesions are probably not warranted. On the other hand, abraded, ischemic peritoneal surfaces clearly lead to adhesions,[12-14] particularly when injury

Table 2 Adhesion Scores for Extent, Type, and Tenacity of Adhesions, Total Adhesion Score, and Histologic Findings in 24 Rabbits: Control Versus Gore-Tex Covered Pelvic Lesions

	Adhesion score (mean ± SD)				Histology	
	Extent	Type	Tenacity	Total	No adhesions	Adhesions
Control	3.4 ± 1.0	3.0 ± 0.9	2.8 ± 0.7	9.1 ± 2.5	5	19
Gore-Tex	1.5 ± 0.7	1.4 ± 0.9	1.4 ± 0.6	4.3 ± 1.8	24	0
P	<0.001[a]	<0.001[a]	<0.001[a]	<0.001[a]	<0.001[b]	

[a] Wilcoxon Signed Rank test.

[b] Chi-square test.

sites are adjacent. The anatomic relationship between pelvic sidewall and adnexal structures predisposes to adhesion formation because the infundibulopelvic and utero-ovarian ligaments suspend the tube and ovary across the pelvic sidewall. Injuries in these areas may particularly benefit from mechanical separation.

Many approaches to mechanical separation of pelvic peritoneal surfaces have been described.[15-17] The barrier approach is an old one and has generally been ineffective because barriers have been either free grafts that become ischemic and promote rather than reduce adhesions or synthetic materials that defeat their purpose by inducing a foreign body reaction. PTFE, however, is widely recognized as being inert. It is not absorbed or otherwise affected by the action of tissue enzymes. PTFE has been used extensively in humans in many forms, from vascular grafts and artificial ligaments to suture material and thin sheets of surgical membrane. Those products differ primarily in their physical characteristics. The widest experience comes from the cardiovascular literature, where PTFE has been found to be nonreactive, nontoxic, and antithrombogenic.[18-21] While the vascular graft is manufactured with a relatively large pore size to encourage tissue attachment and the infiltration of fibrin into its microstructure, SM has an average pore size ≤ 1 micron, minimizing cellular penetration and tissue attachment. Gore-Tex surgical membrane, examined as long as 7 years after placement as a pericardial patch, has been found to induce minimal adhesion formation to either pleural or epicardial surfaces and no foreign body response.[9] SM has also been used experimentally in the rat model to control intra-abdominal adhesions,[22] where it was found to be inert, free of adhesions, and without infectious complications even when used in contaminated surgical cases. The present study suggests that SM might also prove useful in human reproductive surgery, where it could either be left in situ or removed through the laparoscope at second-look surgery.

The rabbit pelvic sidewall/uterine horn model used in this study was purposefully contrived to reproducibly generate adhesions at a high rate in the control lesion. At the same time, it simulates the anatomic relationship that exists between pelvic sidewall and adnexae in the human, where tube–ovary–sidewall are held in proximity by ligamentous attachments. Previous work[23] with various pelvic injuries in the rabbit demonstrated the necessity for approximating injury sites and the mean adhesion score for the control lesion in the current study (9.1 ± 2.5) is very similar to that reported previously for this model (9.0 ± 1.0). Since completion of this study, Goldberg et al.[24] have reported higher adhesion scores for Gore-Tex covered rabbit uterine lesions and concluded that SM was not an effective adjuvant for postoperative adhesion prophylaxis. Their study further illustrates the importance of surgical design because their control lesions generated adhesions at a very low rate, and the Gore-Tex and control lesions were not comparable. SM was held in place with multiple sutures, but no sutures were placed around the control defect, compromising their model.

Adhesion scoring is a subjective process, and the ideal experiment would include blinding. Because the Gore-Tex surgical membrane is clearly visible, blinding was not possible in this study, but the magnitude of the difference between control and Gore-Tex covered lesions and verification of adhesions histologically makes observer bias an unlikely source of error.

In conclusion, the rabbit pelvic sidewall/uterine horn model consistently promotes primary adhesion formation in the control lesion. The interposition of a thin sheet of expanded PTFE (Gore-Tex surgical membrane) significantly reduced the mean adhesion score. The low rate of adhesion formation to the SM-covered lesion was confirmed by histologic examination of the injury sites. Gore-Tex surgical membrane may have a role as an adjuvant in reproductive surgery. Its efficacy in humans remains to be studied.

Acknowledgments. We thank Ms. Gail Cameron, Ms. Jennifer Morgen, and Ms. Margaret Borelli for their expert technical assistance; W. L. Gore & Associates for providing the Gore-Tex surgical membrane; and Ms. Diana Donath for secretarial support.

REFERENCES

1. Diamond MP, Daniell JF, Martin DC, Feste J, Vaughn WK, McLaughlin DS: Tubal patency and pelvic adhesions at early second-look laparoscopy following intraabdominal use of the carbon dioxide laser: initial report of the intraabdominal laser study group. Fertil Steril 42:717, 1984
2. Diamond MP, Daniell JF, Feste J, McLaughlin DS, Martin DC: Pelvic adhesions at early second look laparoscopy following carbon dioxide laser surgical procedures. Infertility 7:39, 1984
3. Pittaway DE, Daniell JF, Maxson WS: Ovarian surgery in an infertility patient as an indication for a short-interval second-look laparoscopy: a preliminary study. Fertil Steril 44:611, 1985

4. Trimbos-Kemper TCM, Trimbos JB, van Hall EV: Adhesion formation after tubal surgery: results of the eighth-day laparoscopy in 188 patients. Fertil Steril 43:395, 1985
5. Adhesion Study Group: Reduction of postoperative pelvic adhesions with intraperitoneal 32% dextran 70: a prospective, randomized clinical trial. Fertil Steril 40:612, 1983
6. Ellis H: The aetiology of post-operative abdominal adhesions: an experimental study. Br J Surg 50:10, 1962
7. Ellis H, Harrison W, Hugh TB: The healing of the peritoneum under normal and pathological conditions. Br J Surg 52:471, 1965
8. Holtz G: Adhesion induction by suture of varying tissue reactivity and caliber. Int J Fert 27:134, 1982
9. Revuelta JM, Garcia-Rinaldi R, Val F, Crego R, Duran CMG: Expanded PTFE surgical membrane for pericardial closure. J Thorac Cardiovasc Surg 89:451, 1985
10. Buckman RF, Buckman PD, Hufnagel HU, Caldwell R: A physiologic basis for the adhesion-free healing of deperitonealized surfaces. J Surg Res 21:67, 1976
11. Levinson CJ, Swolin K: Postoperative adhesions: etiology, prevention and therapy. Clin Obstet Gynecol 23:1212, 1980
12. Buckman RF, Woods M, Sargent L, Gervin AS: A unifying pathogenetic mechanism in the etiology of intraperitoneal adhesions. J Surg Res 20:1, 1976
13. Raftery AT: Effect of peritoneal trauma on peritoneal fibrinolytic activity and intraperitoneal adhesion formation. Eur Surg Res 13:397, 1981
14. Gervin AS, Puckett CL, Silver D: Serosal hypofibrinolysis: a cause of postoperative adhesions. Am J Surg 125:80, 1973
15. Holtz G: Prevention and management of peritoneal adhesions. Fertil Steril 41:497, 1984
16. Stangel JJ, Nisbet JD II, Settles H: Formation and prevention of postoperative abdominal adhesions. J Reprod Med 29:143, 1984
17. Dizerega GS, Hodgen GD: Prevention of postoperative tubal adhesions: comparative study of commonly used agents. Am J Obstet Gynecol 136:173, 1980
18. Matsumoto H, Hasegawa T, Fuse K, Yamamoto M, Saigusa M: A new vascular prosthesis for a small caliber artery. Surgery 74:519, 1973
19. Fujiwara Y, Cohn LH, Adams D, Collins JJ Jr: Use of Goretex grafts for replacement of the superior and inferior venae cavae. J Thorac Cardiovasc Surg 67:774, 1974
20. Smith DE, Hammon J, Anane-Sefah J, Richardson RS, Trimble C: Segmental venous replacement: A comparison of biologic and synthetic substitutes. J Thorac Cardiovasc Surg 69:589, 1975
21. Gazzaniga AB, Lamberti JJ, Siewers RD, Sperling DR, Dietrick WR, Arcilla RA, Replogle RL: Arterial prosthesis of microporous expanded polytetrafluoroethylene for construction of aorta–pulmonary shunts. J Thorac Cardiovasc Surg 72:357, 1976
22. Ratto GB, deBarbieri A, Sacco A, Canepa G, Zaccheo D, Motta G: Use of the polytetrafluoroethylene surgical membrane for control of intra-abdominal adhesions. Italian J Surg Sci 16:165, 1986
23. Diamond MP, Linsky CB, Cunningham T, Constantine B, Dizerega GS, DeCherney AH: Development of a model for sidewall adhesions in the rabbit, and their reduction by an absorbable barrier. Microsurgery 8:197, 1987
24. Goldberg JM, Toledo AA, Mitchell DE: An evaluation of the Gore-Tex surgical membrane for the prevention of postoperative peritoneal adhesions. Obstet Gynecol 70:846, 1987

FERTILITY AND STERILITY
JUNE 1988
vol. 49 no. 6

Modern trends

Editor's corner

Special contribution

Gynecology-endocrinology